



Stapledon Summer Studentship Bursary

Project Completion Report

Supervised by	Dr. Anna Thomson and Prof. Chris Reynolds, University of Reading
Project student	Miss Aayesha Mohammad, University of Roehampton
Dates of studentship	16 th July 2018 – 7 th September 2018
Project title	Determining the effect of botanical composition on the digestibility and nutritive value of multi-species leys for livestock production

BACKGROUND

Recently, the need to reduce the environmental impact of cattle and sheep farming has increased interest in multi-species leys (herbal leys) from within the livestock sectors. The purpose for sowing these leys is to establish a sward that can match the productivity of a fertilised ryegrass pasture but with added resilience to extreme weather, no requirement for inorganic nitrogen (N) fertiliser and a greater ability to provide ecosystem services. Species are selected for inclusion by seed companies based on species persistence and ability to fulfil niche ecosystem functions, however, many of the species within such seed mixtures have not undergone sufficient testing of nutritive value, of which a key aspect is digestibility, despite this being crucial to animal production. The aim of this project was to quantify the nutritional value of species at various maturity stages to enable (i) seed mixture formulation that prioritises high feed value species and (ii) better judgment of correct harvest maturity at the farm level. It is hoped that this will enable better management practice for herbal leys and lead to their increased uptake within the farming community in the future, thereby establishing a widespread network of species-rich pasture. This project built upon a long-term project on herbal leys, led by the University of Reading in collaboration with Duchy College, Rothamsted North Wyke, and Cotswold Seeds (The DiverseForages Project, www.reading.ac.uk/DiverseForages).

METHODOLOGY

The Stapledon Summer Studentship was completed by Miss Aayesha Mohammad, lasting 8 weeks between July and September. Aayesha’s CV can be found in Appendix 2 and a note detailing her experience is included in Appendix 1. During the placement Aayesha helped to established single-

Plot	Species	Functional group	Seed rate g/m ²
1	red clover	Legume	1.5
2	meadow fescue	Grass	3
3	yarrow	Herb	0.25
4	sainfoin	Legume	8.75
5	timothy	Grass	2
6	sheeps parsley	Herb	1
7	sweet clover	Legume	1.5
8	festulolium	Grass	3.5
9	ribgrass	Herb	1
10	white clover	Legume	1
11	tall fescue	Grass	2.5
12	chicory	Herb	1.5
13	alsike clover	Legume	1.5
14	cocksfoot	Grass	2
15	burnet	Herb	2.25
16	Lucerne	Legume	2
17	birdsfoot trefoil	Legume	1

	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18
1																	
2	1					2				3					4		
3	L					G				H					L		
4																	
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Figure 1. A list of species sown and seed rate (left) accompanied by the layout of the successful trial site at Arborfield (right – numbers to the top and left show the scale in metres).

species trial plots at two locations (Sonning and Arborfield) at the University of Reading comprising 17 species of legumes ($n=7$), grasses ($n=5$) and herbs ($n=5$) (Figure 1). An 18th plot of monoculture Ryegrass situated immediately beside these trial plots that was established in the previous year was also utilised. Initial establishment was poor at the Sonning site, which was subsequently discarded, and efforts were focussed on the Arborfield site where plots were hand-weeded and irrigated to ensure the project could continue in a year of severe drought. Grass and herb plots received an application of nitrogen fertiliser equal to 125kg N/ha in July.

Approximately 100g of plant material was harvested from each of the plots at each of two growth stages (Vegetative or Flowering) during August. Six species did not reach flowering stage during the timeframe of the project as a consequence of the drought conditions and therefore only vegetative samples were taken for these. All samples were freeze-dried to a constant weight and ground using a 1mm screen before being analysed for residual dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) in the University of Reading's Metabolism Laboratory using standard methods. *In vitro* true digestibility (IVTD) was determined using the Ankom DAISY incubation method (https://www.ankom.com/sites/default/files/document-files/IVDMD_0805_D200.pdf). A composite sample made up of equal parts of all 18 samples from the vegetative growth stage was also assessed for IVTD to give an indication of whether the plants digestibility was affected by being combined in a mixture. Data were analysed for effects of functional group (grass, legume, herb), maturity (vegetative or flowering) and their interaction using Mixed Models procedure of SAS Version 9.4.

RESULTS AND DISCUSSION

No significant ($P < 0.05$) interactions between plant maturity and functional group were found and therefore results are presented separately for each variable. This suggests that all functional groups experienced similar changes in chemical composition between the two maturity stages. Species harvested at flowering stage contained higher concentrations of OM, NDF, and ADF ($P < 0.001$, < 0.001 and < 0.03 respectively); lower concentrations of CP ($P < 0.002$); and had lower IVTD ($P < 0.002$) than plants at the flowering stage, as would be expected from a plant as the proportion of stem increases relative to leaf during senescence (Table 1). Very high digestibility of plants was observed at the vegetative stage which may reflect stunting of the growth of some species due to the drought conditions (causing them to remain in a shoot-like stage, predominantly comprised of leaves), or an overestimation by the *in vitro* technique. The average IVTD of the vegetative plants incubated alone (877g/kg DM) was similar to that of the mixture sample at 870g/kg DM suggesting that there was no additive effect of combined incubation.

Table 1. The effect of maturity at cutting on chemical composition of all species

Item	Maturity		S.E.M	P-value
	Vegetative	Flowering		
Chemical composition, g/kg DM				
DM, g/kg	259	354	43.6	0.072
OM	876	911	6.7	0.001
CP	203	147	13.6	0.002
NDF	306	406	15.9	0.001
ADF	250	290	18.6	0.021
IVTD	877	743	32.2	0.002

DM = Dry matter; OM = Organic matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = acid detergent fibre; IVTD = *in vitro* true digestibility.

Herbs, legumes and grasses shared similar concentrations of ADF and were equally digestible despite grasses having a higher concentration of NDF than the other two functional groups ($P < 0.001$; Table 2). Herb species contained lower concentrations of organic matter than grasses or legumes ($P < 0.003$), whilst legumes had the highest average CP concentration at 190g/kg DM the effect of functional group on CP concentration was not significant.

Table 2. The effect of functional group on chemical composition

Item	Functional Group			S.E.M	P-value
	Herb	Legume	Grass		
Chemical composition, g/kg DM					
DM g/kg	266	251	403	58.2	0.083
OM	874 ^b	900 ^a	906 ^a	9.2	0.003
CP	172	190	162	18.7	0.293
NDF	300 ^b	287 ^b	480 ^a	21.8	0.001
ADF	269	270	270	13.6	0.998
IVTD	803	821	807	44.2	0.875

DM = Dry matter; OM = Organic matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = acid detergent fibre; IVTD = *in vitro* true digestibility.

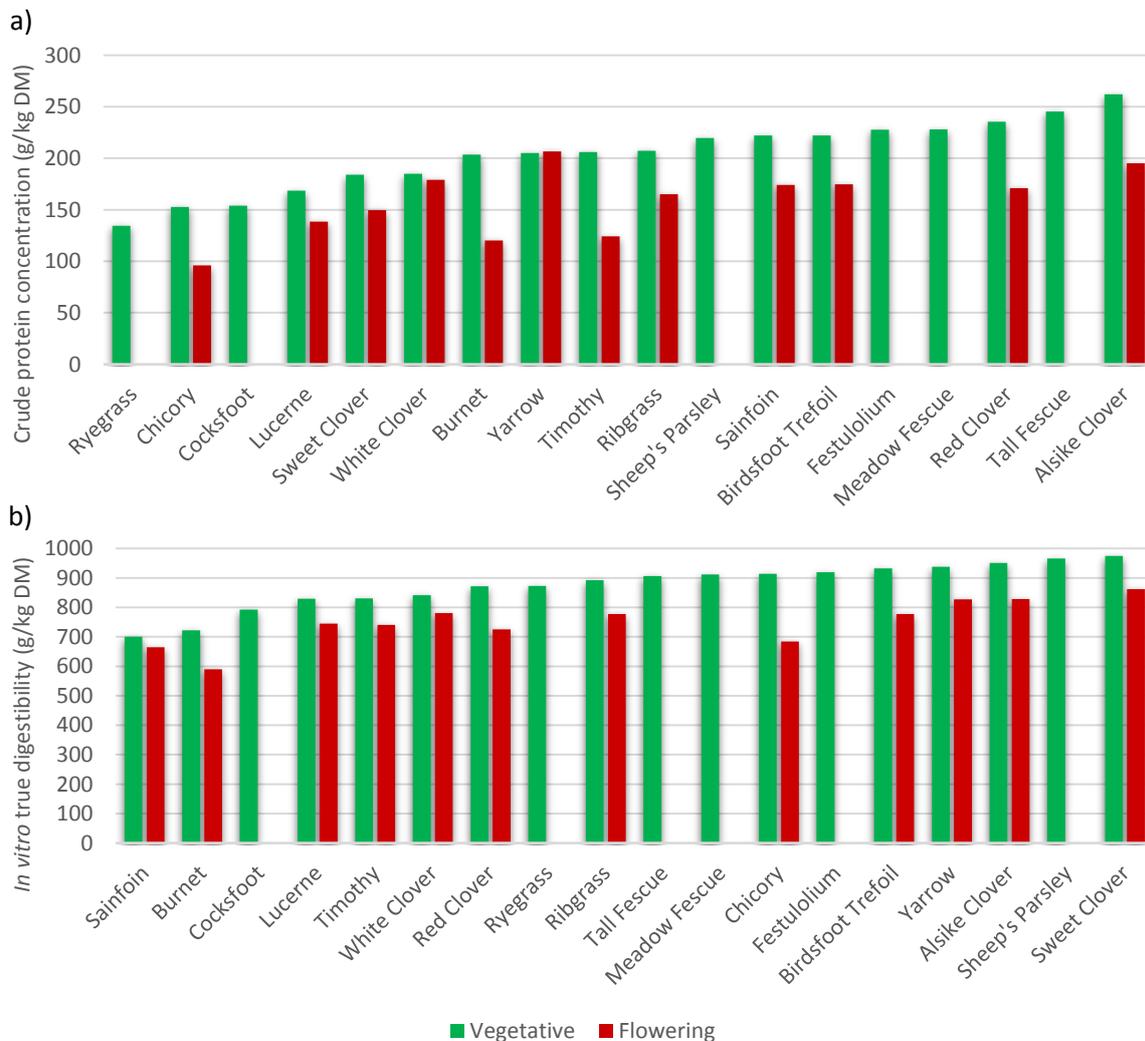


Figure 2. (a) The crude protein concentration and (b) the *in vitro* true digestibility of each species assessed at either vegetative or flowering growth stage.

Although differences were not tested for statistical significance, at the individual species level, alsike clover, tall fescue and red clover contained the highest crude protein concentrations at the vegetative stage (236 – 262 g/kg DM; Figure 2a). Yarrow lost the least crude protein content between vegetative and flowering maturity giving the highest concentration (207 g/kg DM) of any species at the latter stage. The most digestible vegetative species were sweet clover, sheep’s parsley, and alsike clover (Figure 2b), with sweet clover also being the most digestible at flowering stage. If each species were ranked in order of most to least digestible and highest to lowest protein concentration using an average measurement of both maturities, the highest combined ranking species would be alsike clover, sheep’s parsley and birdsfoot trefoil. The lowest ranking based on these data would be perennial ryegrass, lucerne and cocksfoot. The former three species tend to exhibit low persistence in mixed swards indicating a need for new varieties to be produced or imported that are specifically designed to grow in a competitive multi-species environment.

We conclude that, based on this pilot study, species that are largely considered as “bonus” species due to low persistence in mixed leys can be highly nutritious and digestible. Further research under a wider range of conditions, more maturity stages, and over multiple years is required to truly characterise the nutritional value of each species. Ideally, this will then inform which species could be prioritised in breeding programmes specifically targeted at producing groups of plants that are meant to be sown together in a mixture. In this way, the multispecies “herbal” leys of the future might be designed to optimise both animal nutrition and environmental benefit.

ACKNOWLEDGEMENTS

We would like to thank the Stapledon Memorial Trust for providing funding that supported an 8 week student bursary for Aayesha Muhammad and covered the laboratory costs required for this project. We would also like to thank Aayesha for dedicating her time and effort to producing these data. Finally, we would like to recognise the staff of the Centre for Dairy Research who assisted in the establishment of trial plots for this project and Cotswolds Seeds Ltd. who donated the seed required.



Figure 3. Images from the single species trial plots. Top left: Alsike clover. Top right: Chicory. Bottom left: Yarrow; Bottom right: Sweet Clover.

Appendix 1. A note on the benefits to the placement student

Aayesha joined us for 8 weeks in the summer of 2018 in between the second and third years of her zoology degree at the University of Roehampton. One of her main motivations in taking the position was to help her determine whether to pursue a second degree in veterinary sciences or work towards obtaining a PhD after completion of her first undergraduate degree. She had an interest in livestock nutrition and animal behaviour that were both extremely complementary to our work and she took advantage of her stay at the Centre for Dairy Research volunteering to get involved in many aspects of research farm life whilst completing her project work. She learnt a number of new techniques including how to conduct field work with forage trial plots, sample taking of fresh plant material, sample preparation, and a number of different laboratory analyses including using an *in vitro* digestibility technique. She always approached her work with positivity and enthusiasm, asking plenty of questions and contributing her own ideas. In her project work she took responsibility for ensuring all aspects of the study were conducted robustly and on time, and demonstrated an enquiring mind. She hopes to use the data generated by this project to form the basis of her third year dissertation project. We would like to note that Aayesha has been asked to submit her own report on this project which we will forward to the trust later this year. The trial plots established for this project will continue to be managed for the duration of the DiverseForages project and may well provide further opportunities for student research projects in subsequent years.

Appendix 2. CV for placement student Aayesha Muhammad.

Curriculum Vitae

PERSONAL DETAILS

Aayesha Mohammad

03/07/1997

aayeshamd1@gmail.com

Phone Number: 07504 737596

National Insurance No: SN 26 92 78 C

My name is Aayesha and I am currently studying Zoology at the University of Roehampton. I am very fascinated by animals and enjoy working with them which is shown through my previous work experience at veterinary surgeries. While I was there, I cleaned the cages, fed sick animals that had just come out of operations, as well as making sure the animals were calm from the time they came into the veterinary surgery. I believe these practices are vital as cleaning is essential for the animal welfare and to prevent the transmission of any infections. This gave me hands on experience with multiple different types of animals.

I ran a reptile club in my secondary school consisting of a wide range of small animals including: Corn snakes and Leopard Geckos. I also tamed and weaned young snakes and bearded dragons so they were ready for sale and spent my lunch times cleaning their tanks and checking if everything was at its best. It was a great responsibility as I learnt leadership skills and working with people and animals at the same time. It also made me much more confident around animals.

Additionally, I have worked with cows at a milking parlour for 3 weeks which provided me with experience dealing with large animals. I have also worked at a lambing farm for a week where I bottle fed orphaned Lambs and discussed the care of the Ewes as well as cleaning the pens and checking if they had water available at all times. I have also had small animals as pets; for example, Rabbits, so I understand the responsibilities required. Furthermore, I have also worked at Sainsburys for the last one year where I was punctual, gave attention to detail and dealt with members of the public. I have improved various skills, from communication to how to be in a team.

WORK EXPERIENCE

18th Dec to 12th March 2013 **Oakley Veterinary Clinic, Caversham**

Part-time Voluntary on Tuesday's 16:00 to 17:00

Duties:

- Observing operations and helping to set up operations within hygienic standards
- Cleaning and tidying of the practice
- Observing reception and assisting with general information.
- Making sure the animals were well before and after the operation

2nd July to 6th July 2012

St Vincent's Veterinary Surgery, Wokingham
(School work experience)

Duties:

- Shadowing the vet and veterinary nurse
- Observing operations, consultants and helping to set up operations within hygienic standards
- General cleaning and tidying of the practice
- Cleaning cages and providing animals with water and food.

15th August to 1st September 2015

F J & M E Wickens

- Herd management
- Milking
- Feed calves in a timely manner as out-lined or directed by manager
- Application of mastitis treatment in cow's udders
- Screening animals for scour and learning how to treat it

16th April to 20th April 2018

Amners Farm

- Assisting with ewes give birth
- Cleaning lambs after birth
- Feeding orphaned lambs and tube feeding
- Cleaning pens and putting water

25th May to 1st June 2018

Medivet Southfields

- Observing operations and helping to set up operations within hygienic standards
- Cleaning and tidying of the practice
- Observing reception and assisting with general information.
- Making sure the animals were well before and after the operation
- Picking up phone calls
- Laundry

EDUCATION

Highdown School and Sixth Form Centre, Surley Row, Emmer Green, Reading RG4 8LR

GCSE Examinations

GCSE Science Biology	Grade B	GCSE Geography	Grade B
GCSE Science Chemistry	Grade B	GCSE French	Grade D
GCSE Science Physics	Grade B	GCSE Photography	Grade B
GCSE Maths (Methods)	Grade B	GCSE ICT	Distinction
GCSE Maths (Applications)	Grade B	GCSE English	Grade C

AS Examinations Reading College

AS Biology	Grade D
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AS Chemistry	Grade E
AS Maths	Grade D

Reading College: D*D : Level 3 BTEC Applied Science Second year

Current Education: University of Roehampton, Zoology (Second year)

WORK HISTORY

18th Dec to 12th March 2013 **Argos Limited, Oxford Road Reading**

Customer Advisor Part-time

Duties:

- Serving customers at the till's including handling customer payments and refunds
- Served at collection point delivering products to customers
- Advising customers about various products that suits their requirements.
- Distributing Argos catalogues

31st October 2016
to January 2018

Sainsburys, Estate House, 225-231 Upper Richmond Road, London, SW15 6SJ

Customer Advisor Part-time

Duties:

- Serving customers at the till's including handling customer payments and refunds
- Code checking
- Cleaning floors

- Replenishing goods on the shelf

REFERENCES

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